DIVISION OF INTRAMURAL RESEARCH

NAEHS COUNCIL UPDATE

SEPTEMBER, 2001

DIR Recruitments

Scientific Director
After a national search, Dr. Lutz Birnbaumer has been selected to fill position of the Director, Division of Intramural Research,NIEHS. Dr. Birnbaumer is currently Professor and Chair of the Department of Molecular, Cell and Developmental Biology, and Professor of Anesthesiology and Biological Chemistry at the University of California - Los Angeles, and a full member of the Institute of Molecular Biology, the Brain Research Institute and the Jonsson Comprehensive Cancer Center at UCLA.

Chief, Laboratory of Pulmonary Pathobiology
After a national search, Dr. Steven Kleeberger has been selected to be Chief of the Laboratory of Pulmonary Pathobiology (LPP). The LPP is engaged in research on the biology of the respiratory tract system at the cellular, biochemical and molecular level to develop a better understanding of pathogenetic mechanisms involved in development of airway diseases. Dr. Kleeberger is currently a Professor in the Department of Environmental Health Sciences, Johns Hopkins University.

Tenure-Track Neuroscientist
After a national search, the Division of Intramural Research has appointed Dr. Serena Dudek to a Tenure-Track Investigator position in the Laboratory of Toxicology to conduct independent research that will complement or expand ongoing activities in the neurosciences and signal transduction. Dr. Dudek is currently a Senior Staff Fellow with Dr. R. Douglas Fields in the Laboratory of Developmental Neurobiology, National Institute of Child Health and Human Development, NIH. Dr. Dudek's research is focused on signal transduction processes associated with long-term depression and potentiation in hippocampal neurons.

Staff Scientist-Veterinary Pathologist
The Laboratory of Environmental Pathology is seeking a highly motivated Toxicologic Pathologist experienced in rodent toxicology and carcinogenicity studies to work within the National Toxicology Program (NTP). The successful candidate will be involved primarily in the management and oversight of the pathology peer review (evaluation) and interpretation and reporting of the data. Also the candidate will be expected to identify and pursue special projects that will advance the understanding of various biological endpoints. The search committee, chaired by Dr. Gary Boorman (National Toxicology Program), is reviewing applications.

Staff Scientist-Knockout Core Facility Manager
The Laboratory of Reproductive and Developmental Toxicology has re-opened the search for a Staff Scientist with expertise in mouse molecular genetics or a related discipline to serve as the Head of the Transgenic Knockout Core Facility. The successful applicant will generate mutant mice using embryonic stem cell technologies, plan and provide scientific oversight in the conduct of targeting vector design, and serve as a resource expert on mouse genetics and embryology. Dr. Mitch Eddy, Laboratory of Reproductive and Developmental Toxicology, is the chair of the search committee.

Tenure-Track Epidemiologist
A national search is being conducted for a tenure-track investigator who will develop an independent research program in noncancer chronic disease epidemiology, with emphasis on the potential environmental causes of neurological diseases and dysfunctions in humans such as Parkinson's disease, Alzheimer's disease and multiple sclerosis. Individuals with the ability to integrate basic and molecular biology, genetics, toxicology, exposure assessment and epidemiology are sought. Negotiations are currently underway with the leading candidate.

Tenure-track Molecular Toxicologist
The Laboratory of Computational Biology and Risk Analysis is conducting a national search for a tenure-track researcher to develop an independent research program in molecular toxicology focusing on mechanisms of carcinogenicity and toxicity initiated through ligand-receptor interactions. A search committee, chaired by Dr. Douglas Bell, Laboratory of Computational Biology and Risk Analysis, is currently evaluating applications.

Chief, Laboratory of Toxicology
The Environmental Toxicology Program plans to conduct an international search to recruit a senior research toxicologist to serve as Chief of the Laboratory of Toxicology with tenure. Priority will be given to applicants with demonstrated ability to foster effective utilization of molecular technology in the research of the Laboratory, is internationally recognized as an expert in the field of toxicology, and possess a level of managerial and executive ability to create an atmosphere for maximum creativity and scientific productivity. Priority will be given to researchers whose primary interests are in developmental and/or reproductive toxicology with a focus on the molecular basis for environmental causes of disfunction in these areas. Dr. John Pritchard, Chief, Laboratory of Pharmacology and Chemistry, chairs the search committee.

Tenure-track X-ray Crystallographer
The Laboratory of Structural Biology is conducting a nationwide search for a tenure-track X-ray crystallographer to conduct independent research on the structure of proteins, especially those involved in determining biological responses to stress. A search committee chaired by Dr. John Drake, Chief, Laboratory of Molecular Genetics, will start reviewing applications soon.

Tenure-Track Molecular Geneticist
The Laboratory of Molecular Genetics is conducting a nationwide search for a tenure-track molecular geneticist to conduct independent research preferably in the area of genomic stability. Dr. Perry Blackshear, Director, Office of Clinical Research, is the chair of the search committee.

Staff Scientist--Epidemiology
The Epidemiology Branch is conducting a national search to identify a Staff Scientist to help develop a large longitudinal cohort study of environmental effects on children's health and development as part of a federal multi-institution effort. The Staff Scientist will be a member of the interagency coordinating committee responsible for planning and coordinating the proposed study. Duties will include selection of the hypotheses and design of pilot studies in conjunction with the study's working groups, and serving as the project officer on the resulting contracts for the pilot studies. Dr. Beth Gladen, Epidemiology Branch, chairs the search committee.

Tenured Principal Investigator—Signal Transduction

The Laboratory of Signal Transduction is conducting a national search for a tenured Principal Investigator with expertise in G protein receptor signaling andtrafficking. The search committee, chaired by Dr. Kenneth Korach, Chief, Laboratory of Reproductive and Developmental Toxicology, is reviewing applications.

New DIR Recruits

Dr. Geoffery Mueller
Structural Biology Staff Scientist--Nuclear Magnetic Resonance
Dr. Mueller received his Ph.D. from the University of Virginia in 1998 Biophysics from the University of Virginia. His thesis research focused on determining the structure of the potent dust mite allergen Derp 2 using NMR methods. Dr. Mueller then moved to a post-doctoral research position at theUniversity of Toronto and the Hospital for Sick Children working jointly for Lewis Kay and Julie Forman-Kay. There his research focused on developing new NMR techniques that would allow the solving of the structures of larger proteins. This work culminated in the determination of the structure of a 42 kDa protein, which is currently the largest single chain protein solved by NMR spectroscopy.

Dr. Mueller's current role in the Laboratory of Structural Biology is primarily to support research projects on the structural determination of proteins by NMR. Current ongoing projects involve characterizing structural and dynamic properties of DNA polymerases, including E. coli DNA polymerase II, and HIV Reverse transcriptase and its domains. Dr. Mueller also plans to begin work on his own projects in the field of allergen / asthma research.

Publications

1. G.A. Mueller, W.Y. Choy, N.R. Skrynnikov, and L.E. Kay. A method for incorporating dipolar couplings into structure calculations in cases of (near) axial symmetry of alignmment. J. Bio. NMR 2000; 18:183-188.
2. G.A. Mueller, W.Y. Choy, D. Yang, J.D. Forman-Kay, R.A. Venters, and L.E. Kay. Global folds of proteins with low densities of NOEs using residual dipolar couplings: Application to the 370 residue maltodextrin binding protein. J. Molec. Biol. 2000; 300:197-212.
3. G.A. Mueller, A.M. Smith, M.D. Chapman, G.S. Rule, and D.C. Benjamin. Hydrogen Exchange Nuclear Magnetic Resonance Spectroscopy Mapping of Antibody Epitopes on the House Dust Mite Allergen Der p 2 ; J. Biol. Chem. 2001; 276: 9359-9365.
4. G.A. Mueller, D.C. Benjamin, G.S. Rule. Tertiary Structure of the Major House Dust Mite Allergen Der p 2 Determined by NMR Methods: Sequential and Structural Homologies. Biochemistry 1998; 37: 12707-12714
5. G.A. Mueller, A.M. Smith, D.C. Williams Jr., G.A.J. Hakkaart, R.C. Aalberse, M.D. Chapman, G.S. Rule, and D.C. Benjamin. Expression and Secondary Structure Determination by NMR Methods of the Major House Dust Mite Allergen Der p 2. J. Biol. Chem. 1997; 272: 26893-26898.
6. J. Schuurman, G.J. Perdok, G.A. Mueller, and R.C. Aalberse. Complementation of Der p 2 induced histamine release from human basophils sensitized with monoclonal IgE: not only IgE, but also IgG antibodies directed to a non-overlapping epitope of Der p 2. J. of Allergy and Clin. Immunol. 1998; 101: 404-9.
7. J. Schuurman, G. J. Perdok, G. A. Mueller, D. C. Benjamin, K. Y. Tan, M. D. Chapman, and R.C. Aalberse. Mouse/human chimeric IgG1 and IgG4 antibodies directed to the house dust mite allergen Der p 2: Use in quanitfication of allergen specific IgG. Clin. Exp. Allergy, 1997; 27: 1095-1102.

8. N.K. Goto, K.H. Gardner, G.A. Mueller, R.C. Willis, and L. Kay. A Robust and Cost-effective Method for the Production of Val, Leu, Ile (d1) Methyl Protonated 15N-, 13C-, 2H- Labeled Proteins. J. Bio. NMR 1999; 13: 369-374.
9. D. Yang, R.A. Venters, G.A. Mueller, W.Y. Choy, and L.E. Kay. TROSY-based HNCO pulse sequences for the measurement of 1HN-15N, 15N-13CO, 1HN-13CO, 13CO-13CA, and 1HN-13CA dipolar couplings in 15N, 13C, 2H labeled proteins. J. Bio NMR, 1999; 14(4):333-343.
10. N.R. Skrynnikov, N.K. Goto, D. Yang, W.-Y. Choy, J.R. Tolman, G.A. Mueller, and L.E. Kay. Oreinting Domains in Proteins Using Dipolar Couplings Measured by Liquid-state NMR: Differences in Solution and Crystal Forms of Maltodextrin Binding Protein Loaded with beta-cyclodextrin. J. Mol. Biol. 2000, 295: 1265-1273.
11. W.-Y. Choy, M. Tollinger, G. A. Mueller, and L. E. Kay. Direct refinement of high molecular weight proteins against residual dipolar couplings and corbonyl chemical shift changes upon alignment: An application to maltose binding protein. J. Bio. NMR, Submitted

Awards and Honors for DIR Scientists

Dr. Joe Haseman (Biostatistics Branch) was awarded the NIH Director's award.

Dr. Larry Lazarus (Laboratory of Computational Biology and Risk Analysis) was a Special Lecture, 32nd Meeting of the International Narcotics Research Conference, Helsinki, Finland July 15-19, 2001. He was also a Plenary Lecturer at the 4th Symposium on Frontiers in Protein Chemistry and Biotechnology, Chendge, China, August 16-20, 2001.

Dr. Robert Maronpot (Chief, Laboratory of Experimental Pathology) was elected as President of the Society of Toxicologic Pathology effective June 1, 2001.

Dr. Christopher Portier (Associate Driector, National Toxicology Program and Chief, Laboratory of Computational Biology and Risk Analysis) was elected a Fellow of the International Statistical Institute.

Dr. John Pritchard (Chief, Laboratory of Pharmacology and Chemistry) was elected Editor for the Comparative Physiology portion of the American Physiological Society's Regulatory, Integrative, and Comparative Physiology as of August 1, 2001.

Dr. Allen Wilcox (Epidemiology Branch) will give the keynote address September 1st at the Annual Meeting of the International Society of Environmental Epidemiology in Garmisch, Germany.

Dr. Darryl Zeldin (Laboratory of Pulmonary Pathology) received the Lightspan Study Web Academic Excellence Award.

DIR RESEARCH HIGHLIGHTS FOR 2001

A protein found in patients with Alzheimer's disease can disrupt brain signals and therefore may contribute to the memory losses of Alzheimer's disease

NIEHS scientists have demonstrated in rat brain that the major protein of these plaques binds to a receptor in the brain, thus blocking the signals, or currents, that are thought to be involved in learning and memory. This work for the first time establishes this functional link between the plaques seen at autopsy and the failure in brain functioning. The Beta-amyloid peptide blocks the function of a key signaling receptor, the nicotinic acetylcholine receptor, in the hippocampus -- the seat of memory, motivation and emotion in the brain.

Pettit, D. L., Shao, Z., and Yakel, J. L. (2001) β-Amyloid1-42 Peptide Directly Modulates Nicotinic Receptors in the Rat Hippocampal Slice J. Neurosci. 21, RC120: 1-5.

Risk factors for asthma identified

Risk factors for asthma have generally been considered considering childhood asthma as a single entity. It has recently been appreciated that the etiology of asthma may differ according to the age at onset and persistence into later childhood. Few data have been presented in this way. We found that family history of asthma and allergy was most strongly associated with early onset asthma that persists past early childhood, confirming two previous findings. In a novel analysis, we further found that an early life exposure, maternal smoking in pregnancy, was most strongly related to risk of this most serious asthma entity among children with a parental history of asthma or allergy. This finding suggests that early life exposure may be produce the most long lasting harm in genetically predisposed children. This finding could have implications for the study of other early life exposures in asthma.

London, S. J., Gauderman, W. J, Avol, E., Rappaport, E., Peters, J.M. (2001) Family history and the risk of early onset persistent, early onset transient and late onset asthma. Epidemiology in press.

Health benefits of DNA replication errors

Development of a normal human immune system that is capable of reacting with a vast array of foreign antigens requires that immunoglobulin genes undergo somatic mutation at a rate that may be a billion-fold higher than the average genomic mutation rate. The enzymological basis for this hypermutation process has been sought by immunologists for over 25 years. We have just reported that two recently discovered human DNA polymerases, pol eta and pol kappa, generate errors during DNA synthesis whose type and location match those arising during somatic hypermutation. This suggests that one or both of these enzymes may contribute to the development of the normal human immune system.

Rogozin, I.B, Pavlov, Y.I, Bebenek, K., Matsuda, T. and Kunkel, T.A. (2001) Correlation between hot spots for somatic mutation in immunoglobulin genes and DNA synthesis errors by DNA polymerase η. Nature Immunol. 2: 530-536.

Packaging male chromosomes for special delivery

The chromosomes of sperm are packaged tightly into a complex of DNA and unique proteins called protamines. This results in a hydrodynamically shaped sperm head, and may protect the genetic material from damage and to reprogram genes from the father that are expressed in the early embryo. The genes for mouse protamines 1 and 2 were mutated in mice to study the function of these proteins. It was found unexpectedly that a full amount of both proteins is essential for sperm function. A mutation in only one of the two copies of either gene, causing reduction by one half in the amount of protein, led to defects in DNA compaction and male infertility. Protamine 2 deficiency correlates with infertility in humans, suggesting that single-copy mutations in these or other essential sperm proteins may be a cause of infertility in men with apparently normal sperm production.

Cho, C., Willis, W.D., Goulding, E.H., Jung-Ha, H., Choi, Y.-C., Hecht, N.B., Eddy, E.M. (2001) Haploinsufficiency of protamine-1 or -2 causes infertility in mice. Nature Genetics 28: 82-86.

Importance of tumor necrosis factor α (TNF) receptors to the development of the TTP-deficiency syndrome in mice

When mice are deficient in TTP, they develop a systemic syndrome of inflammation, that includes destructive arthritis, autoimmunity, weight loss, and proliferation of normal white cells (granulocytes) to abnormal levels, a process known as myeloid hyperplasia. These investigators showed that most aspects of this syndrome were prevented by interbreeding these mice with mice deficient in both types of TNF receptors, thus implicating TNF in the development of most aspects of the TTP-deficiency syndrome. However, they also showed that the myeloid hyperplasia was contributed to by the oversecretion of granulocyte-macrophage colony-stimulating factor, whose mRNA is also more stable in the absence of TTP.

Carballo, E. and Blackshear, P.J. (2001) Roles of tumor necrosis factor α receptor subtypes in the pathogenesis of the tristetraprolin-deficiency syndrome. Blood in press.

The DDT metabolite DDE in pregnant mothers is associated with increased risk of premature birth

NIEHS investigators have found that in the 1960s pregnant U.S. mothers exposed to the DDT metabolite DDE were more likely to give birth prematurely, and to babies who were unusually small. Premature birth is a major risk factor for infant mortality. Because use of DDT for malaria control is ongoing in 25 countries, the new findings should affect cost-benefit considerations regarding use of pesticides in those countries.

Longnecker, M.P., Klebanoff, M.A., Zhou, H., and Brock, J.W. (2001) Association between maternal serum concentration of the DDT metabolite DDE and preterm and small-for-gestational-age babies at birth. Lancet 358: 110-114.

Database developed for handling and providing initial analyses of microarray data
MAPS (MicroArray Project System), developed for the management and interpretation of microarray gene expression experiment information and data, is a functional database used for storing, compiling and analyzing significant microarray results. This relational database features a web interface to cDNA microarray project information, validate replicate gene expression experimental results, and query gene expression data based on gene classifications of interest. The development of this database has been key in a project where the goal is to validate the "toxicogenomic strategy" by demonstrating that compound classification and correct prediction of unknowns can be accomplished using gene expression arrays.

Bushel, P.R., Hamadeh, H., Bennett, L., Sieber, S., Martin, K., Nuwaysir, E.F., Hayes, K., Reynolds, K., Paules, R., and Afshari, C.A. (2001) MAPS: A MicroArray Project System for Gene Expression Experiment Information and Data Validation. Bioinformatics 17: 564-565.

The *delitto perfetto* approach for gene modification in yeast provides for rapid site-directed change by oligonucleotide based recombination
High throughput analysis of gene function, identification of consequences of disease gene defects, and gene modification to address mechanisms of genome stability is now possible with a system developed in yeast. Rather than traditional, site-directed methods that involve considerable cloning and DNA sequencing, the *delitto perfetto* system provides opportunities to conveniently modify natural chromosomes, cloned human genes, or even yeast artificial chromosomes containing large segments of human DNA. Changes are accomplished by simply adding easily designed oligonucleotides to cells that contain an appropriate DNA cassette in the region to be mutated and counterselecting for loss of the cassette.

Storici, F., Lewis, K. L., and Resnick, M. A. (2001) *In vivo* site-directed mutagenesis using oligonucleotides: a versatile approach based on recombination in yeast. Nature Biotech., in press.
Storici, F., Resnick, M.A. and Lewis, K.L. A versatile system for *in vivo* site-directed mutagenesis with oligonucleotides. Patent filed July 2001.

Retinoid-related orphan receptor gamma (RORγ) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis
To identify the physiological function of the nuclear orphan receptor RORγ, we generated RORγ -/- mice deficient in the expression of RORγ. We showed that these mice do not have lymph nodes suggesting that RORγ is essential for lymph node development. In addition, thymocytes undergo accelerated programmed cell death indicating a role for RORγ in the regulation of thymopoiesis. In addition, RORγ -/- mice are highly susceptible to T-cell lymphoma development. These results suggest important roles for RORγ in lymphoma development and in the control of several immune functions.

Kurebayashi, S., Ueda, E., Sakaue, M., Patel, D.D., Medvedev, A., Zhang, F., and Jetten, A.M. (2000) Retinoid-related orphan receptor gamma (RORgamma) is essential for lymphoid organogenesis and controls apoptosis during thymopoiesis. Proc. Natl. Acad. Sci. U S A. 97: 10132-10137.

A collection of expressed sequence tags (ESTs) from *Xenopus laevis*

Xenopus laevis, the South African clawed frog, has been an important experimental animal for decades that is used for the study of early development. Its developing embryos are also frequently used in toxicology studies. To facilitate analysis of gene expression patterns in normal development and development perturbed by toxins, these investigators created and sequenced a large collection of ESTs from unfertilized eggs, a collection that represents the mother frog's genetic contribution to the first several hours of development. This collection has formed the basis for ongoing efforts to use "gene-chip" analysis to evaluate subtle effects of environmental toxins on development.

Blackshear, P. J., Lai, W. S., Thorn, J. M., Kennington, E. A., Staffa, N. G., Moore, D. T., Bouffard, G. G., Beckstrom-Sternberg, S. M., Touchman, J. W., Bonaldo, M. F., and Soares, M. B. (2001) The NIEHS *Xenopus* maternal EST project: interim analysis of the first 13,879 ESTs from unfertilized eggs. Gene. 267: 71-87.

Drug treatment of lead-exposed children does not improve psychological test scores

NIEHS researchers have demonstrated that Succimer, using a drug to decrease the concentration of lead in blood cannot reverse the IQ damage associated with the lead exposure. Although drug treatment at age two with Succimer lowered blood lead faster than placebo, as expected, it did not improve scores on psychological, behavioral and IQ tests when the children were followed until age five. The results of the trial show clearly that treatment after the fact does not undo the damage among 5 year olds. The study was large enough to have detected an improved IQ score of less than 3 points, and no such improvement was seen. Thus, it is critical to prevent children from being exposed to lead in the first place.

Rogan, W.J., Dietrich, K.N., Ware, J.H., Dockery, D.W., Salganik, M., Radcliffe, J., Jones, R.L., Ragan, N.B., Chisholm, J.J., and Rhoads, G.G. (2001) The effect of Chelation therapy with Succimer on neuropsychological development in children exposed to lead. New Engl. J. Med. 344: 1421-1426.

Women's fertile days even less predictable than previously thought

According to clinical guidelines, the average woman's fertile days are expected to fall between the tenth and 17th days of her menstrual cycle. However, this assumes that ovulation always occurs on the 14th day of the cycle. Although ovulation on day 14 is the average, ovulation is a highly unpredictable event from cycle to cycle. Even women with regular cycles are not able to predict their ovulation day with precision. NIEHS scientists found that more than 70% of women have fertile days before day ten or after day seventeen. Without using some method to monitor their ovulation, there are very few days of the menstrual cycle during which the chance of conceiving is zero.

Wilcox, A.J., Dunson, D., and Baird, D.D. (2000) The timing of the fertile window in the menstrual cycle: day-specific estimates from a prospective study. Brit. Med. J. 321: 1259-62.

The human DNA polymerase – Being wrong for the right reasons?

DNA polymerase iota is the only human polymerase that regularly violates normal Watson-Crick base pairing rules. Unlike other polymerases, it prefers to match thymine with guanine rather than adenine and has thus been considered to be "error-prone". We now discovered that pol iota has a novel enzymatic activity called 5′-dRPase, which removes damage from the 5′ end of a broken DNA strand. This result and additional data suggest that pol iota could participate in specialized types of repair of DNA damage resulting from environmental stress. Among several possibilities, one particularly intriguing hypothesis is that by pairing guanine with thymine, pol iota may prevent misrepair of deaminated 5-methylcytosines, thus actually stabilizing rather than mutating the human genome.

Bebenek, K., Tissier, A., Frank, E.G., McDonald, J.P., Prasad, R., Wilson, S.H., Woodgate, R. and Kunkel, T.A. (2001) 5′-dexoxribose phosphate lyase activity of human DNA polymerase η in vitro. Science 291: 2156-2159.

Simple steps can reduce dust mite allergen exposure in low income, urban homes

Asthma disproportionately affects inner-city, lower socioeconomic status individuals. Dust mite allergen exposure is an important risk factor for asthma development; however the best methods to reduce exposure to this allergen in low income, urban homes remain unknown. NIEHS scientists found that using impermeable mattress/pillow covers in combination with weekly laundering of bedding significantly reduces dust mite allergen exposure in the bed. Moreover, steam cleaning of bedroom carpets together with vacuuming reduces dust mite allergen levels in bedroom floors. Simple, practical interventions may provide effective means for reducing dust mite allergen exposure in high risk environments.

Vojta, P. J., Randels, S., Stout, J., Muilenberg, M., Burge, H., Mitchell, H., O'Conner, G. and Zeldin, D. C. (2001) Effects of physical interventions on group I house dust mite allergen levels in carpet, bed, and upholstery in inner city homes. Env. Health Perspect. in press.

Genetic polymorphisms affect metabolism of the anticancer drug taxol used in treatment of breast cancer

Two genetic polymorphisms were discovered in a drug-metabolizing enzyme CYP2C8 which is responsible for metabolism of the anticancer drug taxol in humans. One polymorphism (named CYP2C8*3) causes two amino acid substitutions in the protein. The recombinant human enzymes have been expressed *in vitro*, and the polymorphism decreases metabolism of taxol dramatically. Genetic tests have been devised to test for these polymorphisms in human blood,

and these tests will be used to examine whether these polymorphisms affect blood levels of taxol and its toxicity in breast cancer patients who are being treated therapeutically with taxol.

Dai, D., Zeldin, D., Blaisdell, J. A., Chanas, B., Coulter, S. J., Ghanayem, B. I., and Goldstein, J. A. (2001) Genetic polymorphisms of human CYP2C8 and their effects on the metabolism of paclitaxel and arachidonic acid. In press, Pharmacogenetics.

A new signaling mechanism for thyroid hormone.

Thyroid hormone is essential for normal brain development. In humans thyroid hormone reductions produced by iodine deficiency or by chemical exposure to environmental toxins results in severe neurological impairment, but there is no molecular mechanism to explain these effects. We have discovered that thyroid hormone inhibits secretion from a rat pituitary cell line by stimulating a specific class (KCNH2) of potassium ion channels through a Rac dependent process. In view of the recent demonstration by others that the Rac GTPase is essential for neurite outgrowth in the embryonic nervous system, our discovery linking thyroid hormone action to Rac provides a direct molecular mechanism for the deleterious health effects of thyroid hormone deficiency.

Storey, N., O'Bryan, J., and Armstrong, D. L. (2001) Potassium channel stimulation by nuclear hormone family receptors through a Rac-dependent signaling pathway in a rat anterior pituitary cell line GH4C1. J. Physiol., in press.

Molecular mechanism of mitochondrial toxicity by AIDS drugs reveals several modes of inhibition

Mitochondrial toxicity can result from antiviral nucleotide analog therapy used to control HIV-1 infection. The Anti-HIV nucleoside analogs 2',3'-dideoxy-TTP (ddTTP), 3'-azido-TTP (AZT-TP), 2',3'-dideoxy-CTP (ddCTP), 2',3'-didehydro-TTP (D4T-TP), (-)-2',3'-dideoxy-3'-thiacytidine (3TC-TP), and carbocyclic 2',3'-didehydro-ddGTP (CBV-TP) were examined for their ability inhibit DNA synthesis by the human mitochondrial DNA polymerase. Dideoxynucleotides and D4T-TP were utilized by the mitochondrial DNA polymerase in vitro as efficiently as natural deoxynucleotides, whereas AZT-TP, 3TC-TP and CBV-TP were only moderate inhibitors of DNA chain elongation. Inefficient excision of dideoxynucleotides, D4T, AZT, and CBV from DNA predicts persistence in vivo following successful incorporation. In contrast, removal of 3'-terminal 3TC residues was 50% as efficient as natural 3'-termini. Finally, we observed inhibition of exonuclease activity by concentrations of AZT-monophosphate known to occur in cells. Thus, although their greatest inhibitory effects are through incorporation and chain termination, persistence of these analogs in DNA and inhibition of exonucleolytic proofreading may also contribute to mitochondrial toxicity.

Lim, S. E., and Copeland, W. C. (2001) Differential incorporation and removal of antiviral deoxynucleotides by human DNA polymerase γ. J. Biol. Chem.in Press.

Effect of normal and mutant MARCKS protein on cell adhesion
MARCKS protein has been shown to be critical for the normal development of the brain and retina, and in its absence there is evidence for abnormal migration of developing neurons in the brain. To understand this migration defect better, these investigators studied the effect of mutating this protein on the adhesion of cultured cells to protein- coated surfaces. Expression of the normal protein inhibited cell adhesion; however, when certain critical regions of the protein that control its membrane association were deleted, this effect was lost. These studies may have implications for how MARCKS is used to control migration of brain neurons during development.

Spizz, G., Blackshear, P.J. (2001) Overexpression of the myristoylated alanine-rich c-kinase substrate inhibits cell adhesion to extracellular matrix components. J. Biol. Chem. in press

P450-derived eicosanoids are cytoprotective
CYP2J2 metabolizes arachidonic acid to epoxyeicosatrienoic acids (EETs) which are rapidly metabolized by soluble epoxide hydrolase. NIEHS scientists showed that exposure of endothelial cells to hypoxia-reoxygenation results in significant cell injury and reduced CYP2J2 expression. Importantly, maintenance of CYP2J2 levels, addition of synthetic EETs, or inhibition of soluble epoxide hydrolase attenuates the hypoxia-reoxygenation-induced cell injury. The cytoprotective effects of CYP2J2 appear to be mediated, at least in part, by antioxidant effects.

Yang, B., Graham, L. Falck, J. R., Liao, J. K., and Zeldin, D. C. (2001) Overexpression of cytochrome P450 CYP2J2 protects against hypoxia-reoxygenation injury in cultured bovine aortic endothelial cells. Mol. Pharm. in press.

Cyclooxygenase-derived eicosanoids mediate airway responsiveness to endotoxin
Endotoxin is an important environmental agent that causes airway inflammation and is a risk factor for asthma. NIEHS scientists found that compared to wild type mice, mice deficient in either COX-1 or COX-2 exhibit increased bronchoconstriction following inhaled endotoxin. These changes occur in the absence of an enhanced airway inflammatory response. These data indicate that both COX-1 and COX-2 are important in regulating the functional respiratory responses to endotoxin, but not the inflammatory responses.

Zeldin, D. C., Wohlford-Lenane, C., Chulada, P., Bradbury, J. A., Scarborough, P. E., Roggli, V., Langenbach, R. and Schwartz, D. A. (2001) Airway Inflammation and responsiveness in prostaglandin h synthase-deficient mice exposed to bacterial lipopolysaccharide. Am. J. Resp. Cell Mol. Biol. in press.

Importance of the polyA tail to the turnover of mRNA stimulated by the CCCH tandem zinc finger proteins

Tristetraprolin, or TTP, can bind to certain regions near the end of certain medically important cytokine mRNAs and cause "destabilization" of the mRNAs, resulting in a novel form of control of gene expression that is post-transcriptional. These investigators evaluated the importance of the polyA tail in the "target" mRNA to the ability of TTP to stimulate degradation of that mRNA. They found that the TTP effect could be seen even in the absence of the polyA tail, implying that the mechanism of TTP-mediated mRNA decay does not only involve removal of the mRNA-stabilizing polyA tail from the mRNA.

Lai, W. S. and Blackshear, P. J. (2001) Interactions of CCCH zinc finger proteins with mRNA: tristetraprolin-mediated AU-rich element-dependent mRNA degradation can occur in the absence of a poly(A) tail. J. Biol. Chem.276: 23144-23154.

Protein kinase A signaling may play a role in utmor cell metastasis

NIEHS scientists have found that up-regulation of protein kinase A (PKA) may play a role in increasing cell-cell adhesion in tumor cells. In this case, cell-cell adhesion is mediated by integrins from the beta-1 family, receptors that usually function in cell-substrate adhesion. This is the first indication that PKA may play a role in the regulation of cell-cell adhesion. This finding suggest that adhesive receptor activation through protein kinase A signaling pathways may be important in tumor cell metastasis.

Whittard, J.D. and Akiyama, S.K. (2001) Positive regulation of cell-cell and cell-substrate adhesion by protein kinase A. J. Cell Sci., in press.

Imaging blood-brain barrier function

Capillaries in the brain constitute a barrier to the entry of foreign chemicals, such as, neurotoxins and therapeutic drugs. A critical impediment to understanding transport function in intact brain capillaries is the lack of suitable in vitro techniques that both retain viability and that allow the investigator to measure movement of diffusible solutes across the endothelium. We utilized the optical sectioning capabilities of confocal microscopy to visualize and measure the accumulation of fluorescent drugs within the lumens of freshly isolated capillaries from rat and pig brain. The images show that two ATP-driven xenobiotic export pumps constitute an active barrier to entry into the central nervous system.

Miller, D. S., Knobmann, S. N., Gutmann, H., and Fricker, G. (2000) Xenobiotic transport across isolated brain microvessels studied by confocal microscopy. Mol. Pharmacol., 58:1357-1367.

Coordination of fidelity mechanisms in DNA replication

DNA polymerases achieve fidelity in two ways during DNA replication: they insert correct bases with high accuracy, and most of the rare insertion errors are promptly corrected by a proofreading mechanism. NIEHS scientists have characterized the efficiencies of these two fidelity mechanisms in a model organism that uses a DNA polymerase related to those used by human cells. It turns out that insertion fidelity and proofreading efficiency are closely linked by a process yet to be understood.

Bebenek, A., Dressman, H. K., Carver, G. T., Ng, S., Petrov, V., Yang, G., Konigsberg, W. H., Karam, J. D. and Drake, J. W. (2001) Interacting fidelity defects in the replicative DNA polymerase of bacteriophage RB69. J. Biol. Chem. 276: 10387-10397.

Identification of a novel therapeutic target for treatment of high blood pressure

Cytochrome P450s metabolize arachidonic acid to epoxyeicosatrienoic acids (EETs) which are potent vasodilators. NIEHS scientists showed that soluble epoxide hydrolase (sEH) regulates hydrolysis of these vasoactive epoxides and inhibitors of this enzyme lower blood pressure in spontaneously hypertensive rats but not in normotensive control rats. This study was the first to identify sEH as a novel therapeutic target for blood pressure control.

Yu, Z., Xu, F., Huse, L. M., Morisseau, C., Draper, A., Newman, J. W., Parker, C., Graham, L., Engler, M., Hammock, B. D., Zeldin, D. C., and Kroetz, D. L. (2000) Soluble epoxide hydrolase regulates hydrolysis of vasoactive epoxyeicosatrienoic acids. Circ. Res. 87: 992-998.

A system has been developed for the functional analysis of mutations in the p53 tumor suppressor gene.

The p53 gene is required for regulating many cellular responses to environmental stress, particularly DNA damage. While p53 mutations are commonly associated with tumors, there has been limited opportunity to characterize them and to define the functional consequences of the mutations. Using yeast as an in vivo test tube, an exquisitely sensitive system has been developed that provides for rapid analysis of subtle changes in p53 function. These approaches will be useful in developing therapy strategies as well as evaluating the p53 gene as a target of environmental damage.

Inga, A., Monti, P., Fronza, G., Darden, T. and Resnick, M. A.(2001) Novel p53 mutants exhibiting enhanced transcriptional activation and altered promoter selectivity are revealed using a yeast-based functional assay. Oncogene 20: 501-513.

Inga, A. and Resnick, M. A.(2001) Human p53 mutants that are toxic in yeast, exhibit enhanced transactivation, promoter selectivity, reactivate tumor mutants and can suppress growth of human cells. Oncogene, in press.

Toxicology of AIDS therapeutics

Infection with human immunodeficiency virus (HIV) causes immunodeficiency and leads to acquired immunodeficiency syndrome (AIDS). Majority of AIDS patients die from opportunistic infections. Prophylaxis and treatment AIDS are generally combination therapies of antiretroviral agents and antimicrobial drugs specific for the opportunistic infections. Toxic effects of most anti-HIV drugs and most opportunistic infection drugs are known. However, toxic effects of

some individual drugs and most combination therapies are not well established. The NIEHS under the AIDS research program, is evaluating AIDS therapies for reproductive, developmental and general toxicity and carcinogenicity in rodents. These evaluations may include single therapeutic agents or combination therapies when the toxic potential of these agents in animal models is not available or is incomplete.

Tuberculosis is a common opportunistic infection in HIV positive patients. Rifampicin is a commonly used antituberculosis drug either alone or in combination with other antituberculosis drugs. Combination therapy with antituberculosis drugs such as Rifampicin and AZT is a common procedure for treatment of HIV positive patients suspected of having tuberculosis. Toxic consequences of these combination therapies are not established. Rifampicin alone or AZT alone caused mild hematological toxicity. Rifampicin when administered in combination with AZT markedly increased the hematological toxicity in a mouse model. Detailed information on toxicity of combination therapy of AZT and Rifampicin is reported in the following recent publication.

National Institute of Environmental Health Sciences, 2001. Subchronic Toxicity Study of 3'-Azido-3'-Deoxythymidine (AZT) and Rifampicin Combinations Administered by Gavage to B6C3F1 Mice. NIEHS AIDS Therapeutics Toxicity Report No. 6. NIH Publication 01-4401. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. Research Triangle Park, NC.

P450-derived eicosanoids possess fibrinolytic properties

Cytochrome P450s metabolize arachidonic acid to epoxyeicosatrienoic acids (EETs) in the cardiovascular system. NIEHS scientists have showed that one of the EETs increases endogenous tissue plasminogen activator (t-PA) expression via a mechanism that involves activation of $G\alpha_s$ and increased intracellular cAMP. Overexpression of the human arachidonic acid epoxygenase CYP2J2 in endothelial cells recapitulates these effects. Together these data indicate that CYP2J2-derived EETs play an important role in regulating vascular hemostatis.

Node, K., Ruan, X.-L., Dai, J., Yang, S.-X., Graham, L., Zeldin, D. C. and Liao, J. K. (2001) Induction of tissue-type plasminogen activator expression by cytochrome p450 epoxygenase-derived eicosanoids. J. Biol. Chem. 276: 15983-15989.

Discovery of a new cytochrome P450 arachidonic acid hydroxylase in brain

NIEHS scientists cloned a new cytochrome P450 (CYP2J9) which is abundant in brain, highly expressed in Purkinje cells and active in the omega-1 hydroxylation of arachidonic acid. The major product of this enzyme (19-HETE) inhibits the activity of P/Q-type Ca^{++} channels which are also abundant in Purkinje cells and involved in triggering neurotransmitter release. Importantly, the expression of this P450 is increased by mercury vapor which is a known neurotoxin. These data indicate that CYP2J9 is regulated by environmental factors and may play important functional roles in the brain.

Qu, W., Bradbury, A., Tsao, C.-C., Maronpot, R., Harry, G. J., Davis, L., Breyer, M. D., Waalkes, M., Parker, C., Falck, J. R., Chen, J., Rosenberg, R. and Zeldin, D. C. (2001) Cytochrome P450 CYP2J9, a new mouse arachidonic acid ω-1 hydroxylase predominately expressed in brain. J. Biol. Chem. in press.

Salmonella hijacks the cells' own signalling mechanisms in order to enter cells.
Certain cells are adapted to accumulate and destroy bacteria by a process known as phagocytosis. The enteric pathogen, Salmonella, tricks cells that are not professional phagocytes to nevertheless use phagocytosis (but in a non-destuctive manner) so as to accumulate the bacteria. Better understanding of the molecular mechansism could lead to improved therapies. In this report, we show how Salmonella hijacks cellular inositol phosphate phosphatases, and we show how this is associated with changes in cell architecture that preceed phagocytosis.

Zhou, D., Chen, L. M., Hernandez, L., Shears, S. B. and Galán, J. E. (2001) A Salmonella inositol polyphosphatase acts in conjunction with other bacterial effectors to promote host-cell actin cytoskeleton rearrangements and bacterial internalization. Molec. Microbiol.. 39: 248-259 2001

The exonuclease of lagging strand DNA polymerase prevents replication-associated DSBs.
DNA replication and processing of intermediates requires coordinated interactions between many proteins. A combination of sophisticated genetic and biochemical approaches has revealed that during DNA replication, sites between ending and starting strands are particularly vulnerable to breakage. The exonuclease function that was considered to be an error correction function within DNA polymerase has now been shown to also serve as a backup for for the 5' flap endonuclease Rad27/Fen1 in processing Okazaki replication intermediate fragments. This function prevents the appearance of genomically destabilizing double-strand breaks. Because of the conservation of genes and systems between yeast and humans, these observations are relevant to understanding factors affecting human genome stability.

Jin, H. J., Obert, R., Burgers, P. M. J., Kunkel, T. A., Resnick, M. A., and Gordenin, D. A. (2001) The 3'→5' exonuclease of DNA polymerase δ can substitute for the 5' flap endonuclease Rad27/Fen1 in processing Okazaki fragments and preventing genome instability. Proc. Nat. Acad. Sci. USA 98: 5122-5127.

Prevention or delay of breast cancer
Breast cancer is one of the most common cancers in women and is the second leading cause of cancer deaths of women in the United States. Animal models are useful for understanding the biology of breast cancer and for evaluation of prevention strategies and therapeutic approaches. Transgenic mouse lines with homologues of human breast cancer oncogenes are available. Diet and its ingredients/components may be the most important environmental/life-style factors contributing to the development of breast cancer. Transgenic mouse line TG.NK with c-*neu* the human breast cancer homologue of *erb*B2 develops palpable mammary tumors after 20 weeks of age. There is approximately 18-week interval between weaning and development of palpable

mammary tumors to evaluate intervention strategies to prevent/delay mammary cancer in TG.NK mouse model. Nonpurified diets, especially fiber-rich cereal ingredient diets appear to be highly beneficial in delaying the development of mammary cancer in TG.NK mouse model. Some components of diet, dietary supplements, vitamin A and its analogues (retinoids) and therapeutic agents such as tamoxifen may delay or prevent mammary cancer. The TG.NK transgenic mouse model could be used to develop and evaluate dietary supplements and therapeutic agent combination intervention strategies to prevent breast cancer.

Melatonin is a neurohormone produced mainly by the pineal gland during the hours of darkness in most mammalian species. Melatonin is considered to be useful for inhibition of induced, transplanted and spontaneous mammary cancer. Melatonin is readily available as a dietary supplement in the United States. Melatonin at 50 mg /kg body weight of the mouse, a nontoxic dose decreased the incidence and markedly delayed the development of mammary cancer in the transgenic TG.NK mouse model.

Diets rich in omega-3 polyunsaturated fatty acid (ω-3 PUFA) from fish oils increased the tumor latency period and decreased the growth of tumors compared to diets rich in ω-6 PUFA from vegetable oils such as corn oil. But, fish oil may decrease platelet aggregation and blood viscosity, and increase erythrocyte deformity. An alternate approach could be to use vegetable oils such as flaxseed oil rich in α-linolenic (C18:3, ω-3) fatty acid, a precursor of 20:5 and 20:6 ω-3 fatty acids. Table grade flaxseed oil with approximately 60% of fatty acids as α-linolenic (C18:3, ω-3) fatty acid is readily available as a dietary supplement in the United States. The α linolenic (C18:3, ω-3) fatty acid provided by flaxseed oil appears to delay the growth of mammary cancer if the ω-6: ω-3 PUFA ratio of fat consumed is closer to 1, but may promote tumor growth if the ratio is 0.3 or lower. To achieve a ω-6: ω-3 PUFA To achieve a ω-6: ω-3 PUFA ratio of 1 in the diet, more than 50% of the fat consumed may have to be flaxseed oil and fish oil.

Rao, G.N., Ney, E., and Herbert, R.A. (2000) Effect of melatonin and linolenic acid on mammary cancer in transgenic mice with c-neu breast cancer oncogene. Breast Cancer Research and Treatment. 64: 287-296.

Are plasticizers in the environment making you infertile?

Chemicals in the environment that disrupt endocrine and reproductive function are of concern to the public. One chemical of concern is di-(2-ethylhexyl) phthalate, which is manufactured in the amount of a million tons per year to enhance plastics and then ends up as a common environmental contaminant. Di-(2-ethylhexyl) phthalate disrupts endocrine and reproductive function in model species by preventing ovulation leading to infertility. NIEHS scientists report that the active metabolite of this phthalate affects the ovary by altering levels of aromatase, an enzyme responsible for making the female hormone estradiol. Because the steps involved in hormone production are very similar in rodents and humans, this work may help in understanding how this chemical affects reproductive function in women.

Lovekamp, T. N. and Davis, B. J. (2001) mono- (2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. Tox. Appl. Pharmacol. 172: 217-224.

First use of radial TAR cloning from the murine genome

Transformation-associated recombination (TAR) cloning allows entire genes and the adjacent DNA regions from the chromosome to be specifically, accurately and quickly isolated. NIEHS scientists reported the first example of radial TAR cloning from the mouse genome by isolating the gene in Tg.AC mice that is responsible for the skin tumors this mouse develops following exposure to carcinogens.

Humble, M.C., Kouprina, N., Noskov, V.N., Graves, J., Garner, E., Tennant RW, Resnick, M.A., Larionov, V., and Cannon, R.E. (2000) Transformation-associated recombination cloning from the mouse genome: isolation of Tg.AC transgene with flanking DNAs. Genomics 70: 292-299.

Development of an experimental model for benzene induction of leukemia and/or lymphoma in genetically altered rodents.

Benzene is an important chemical compound with many uses in chemistry and production of goods as well as fuels for power. However, due to such large-scale manufacture and use of petroleum based product containing benzene compound has become ubiquitous in our environment. Long term exposure to benzene is associated with the development of leukemia and lymphoma. Understanding benzene toxicity has been hampered by the lack of rodent models to investigate its toxicity, complex metabolism, and identification of the by-product that are toxic and may cause leukemia or lymphoma (blood diseases that are continuing to increase in our population). Using deficiencies or overexpression of genes (oncogenes and tumor suppressor genes) known to be involved in human cancers, we have been able to show that low levels of exposure may cause leukemias and/or lymphomas in rodents. This will help us in further developing research to determine both the mechanism of the cause of the cancer and what levels of exposure must be avoided in order to reduce the risk to humans.

French, J.E. and Saulnier, M. (2000) Benzene Leukemogenesis: An Environmental Carcinogen Induced Tissue Specific Model of Neoplasia using Genetically Altered Mouse Models. J Toxicol Env Health, 61:101-103.

Healy, L.N., Pluta, L.J., James, R.A., Janszen, D.B., Torous, D., French, J.E., and Recio, L. (2001) Induction and time-dependent accumulation of micronuclei in peripheral blood of transgenic p53 +/- mice, Tg.AC (v-Ha-ras) and parental wild-type (C57BL/6 and FVB/N) mice exposed to benzene by inhalation. Mutagenesis 16: 163-168.

Genistein, a plant estrogen found in soy products, increases cancer in animals.

A new animal study reports that treatment with genistein during development causes cancer of the reproductive tract later in life. NIEHS scientists have observed an increase in cancer of the uterus in

aged female mice treated with genistein for only 5 days after birth. Humans are exposed to high levels of genistein during development through the use of soy-based infant formulas and soy products marketed specifically to appeal to children. The use of soy-based infant formulas in the absence of medical necessity and the marketing of soy products designed to appeal to children should be closely examined.

Newbold, R. R., Banks, E. P., Bullock, B., and Jefferson, W. N. (2001) Uterine adenocarcinoma in mice treated neonatally with genistein. Cancer Res. 61: 4325-4328.

Dietary phytoestrogens and the incidence of spontaneous vulvar carcinomas.
NIEHS scientists examined the effect of dietary phytoestrogens in 129/J mice using three natural ingredient diets and two purified diets containing predetermined levels of daidzein and genistein. At three months, the incidence of vulvar carcinomas in mice fed the soybean protein diet was significantly ($p<0.05$) increased over that of mice fed the NIH-31 diet or the PMI, #5K96 diet. There was a significant ($p<0.10$) correlation between the total daidzein and genistein levels in the five tests diets and the incidence of vulvar carcinomas in mice.

Thigpen, J.E., Locklear, J., Haseman, J.K., Saunders, H., Grant, M.F., and Forsythe, D.B. (2001) The effects of the dietary phytoestrogens daidzein and genistein on the incidence of spontaneous vulvar carcinomas in 129/J mice. Cancer Detect. Prevent., in press.

Phosphorylation sites and levels on hyperphosphorylated p53 determined.
Using a combination of several isolation techniques combined with mass spectrometric analysis, six specific phosphorylation sites were identified on p53 after expression in the presence of a phosphatase inhibitor. One of these sites, ser46, had not been previously observed. In addition, The relative levels of phosphorylation at a specific site were determined. For example, ser315 was completely phosphorylated in hyperphosphorylated p53, but only phosphorylated to 10-20% in 'normal' p53. We speculate that the multiple phosphorylated forms serve as precursors for distinct subpopulations with different biological activity.

Merrick, B.A., Zhou, W. Martin, K.J., Jeyarajah, S., Parker, C.E., Selkirk, J.K., Tomer, K.B., and Borchers, C.H. (2001) Site-specific phosphorylation of human p53 protein determined by mass spectrometry. Biochemistry 40: 4053-4066, 2001.

Statistical Issues in the Analysis of Low Dose Endocrine Disruptor Data Ealuated
Recently, the NIEHS co-sponsored a meeting to examine data addressing the presence or absence of low-dose effects of endocrine disruptors in specific studies. All invited speakers agreed to provide their raw data in advance of the meeting to a Statistics Subpanel, that was asked to re-evaluate the authors' experimental design, data analysis, and interpretation of experimental results. Dr. Haseman of the NIEHS chaired this subpanel, and a paper summarizing their evaluation of 38 individual studies was recently published. The statistical principles and

issues that are discussed in this paper are not unique to endocrine disruptor studies, and provide important guidelines regarding appropriate experimental design and statistical analysis for other types of laboratory investigations.

Haseman, J. K., Bailer, A. J., Kodell, R. L., Morris, R., and Portier, C. (2001) Statistical issues in the analysis of low dose endocrine disruptor data. Tox. Sci. 61: 201-210.

Telomeric interactions have genetic consequences and can activate repressed genes

In Drosophila telomeric repeat arrays consist of non-LTR retrotransposons, primarily HeT-A, rather than the canonical short repeats found at telomeres in many other species. As in other species, reporter genes inserted into telomeres of Drosophila are repressed. All of the repressed reporter genes that have been tested have inserted into or adjacent to the telomere associated repeat sequence usually found adjacent to the terminal repeat array. Increasing the length of the HeT-A array causes an increase in downstream reporter gene activity under some circumstances, but only when TAS on the homologous telomere is defective. Disruptions of the TAS sequence on one homologous telomere by insertion or deletion cause increased HeT-A mediated transcription of a reporter gene on the other homologous telomere.

Golubovsky, M. D., Konev, A.Y., Walter, M.F., Bliessman, H., and Mason, J. M. (2001) Terminal retrotransposons activate a subtelomeric *white* transgene at the 2L telomere in Drosophila. Genetics 158: 1111-1123.

Characterization of the control of telomeres in Drosophila

In Drosophila, telomeric repeat arrays consist of non-LTR retrotransposons, primarily HeT-A, attached by their oligo(A) tails to the chromosome end, rather than the canonical short repeats found at telomeres in many other species. As in other species, telomere associated sequences, TAS, are present adjacent to the terminal repeat arrays, and reporter transgenes inserted into TAS are repressed and variegate. Models for variegation of genes in centric regions do not account for all of the attributes of telomeric variegation. Rather, a model that does explain all of the published and unpublished data proposes that variegation of telomeric reporter genes is the result of a competition between transcription initiated in the retrotransposon array and silencing from the TAS region. Modulation of this competition regulates transposition of the telomere-specific retrotransposons, and thus telomere length. HeT-A transposition normally occurs with a frequency sufficient to balance the loss of DNA from the chromosome end due to incomplete replication. Two mutations have been discovered that influence the frequency of HeT-A addition onto a chromosome end. One completely eliminates HeT-A additions, the other increases the wild-type frequency about 100X. These mutations are being used to investigate the mechanism of telomere length maintenance in Drosophila.

Mason, J.M., Haoudi, A., Konev, A.Y., Kurenova. E., Walter, M.F., and Biessmann, H. (2000) Control of telomere elongation and telomeric silencing in Drosophila melanogaster. Genetica: 109: 61-70.

Chemical metabolism in genetically altered mice used in short-term carcinogeicity studies is not different from the respective unaltered strain

The use of transgenic animals such Tg.AC and p53+/- mice offers promise as a rapid and sensitive assay for chemical carcinogenesis. Some chemicals are carcinogenic only after they have been altered by metabolism in the animal, therefore it is critical that the transgenic animals retain metabolic capability. By using metabolism studies of model chemicals designed to test various metabolic pathways and using molecular biology techniques to measure enzyme production, we have reached the conclusion that the ability of the transgenic mice to metabolize chemicals is not compromised by the altered genomes.

Sanders, J.M., Burka, L.T., Chanas, B., Matthews, H. B. (2001) Comparative xenobiotic metabolism between Tg.AC and p53+/- genetically altered mice and their respective wild types, Tox. Sci. 61: 54-61.

Mutation rates in a microbe from hell

All microbes studied to date maintain the same rate of spontaneous mutation, about 0.003 mutations per genome per replication, whereas riboviruses, animals and plants have higher rates. Can an organism living in an intrinsically highly mutagenic environment maintain this low rate? The archaeon Sulfolobus acidocaldarius grows happily at pH 3.5 at 70°C, conditions that rapidly damage DNA. Nevertheless, this microbe retains the characteristic low mutation rate seen in a bacterium, a yeast a fungus, and several DNA viruses.

Grogan, D. W., Carver, G. T. and Drake, J. W. (2001) Genetic fidelity under harsh conditions: analysis of spontaneous mutation in the thermoacidophilic archaeon Sulfolobus acidocaldarius. Proc. Natl. Acad. Sci. USA 98, in press..

Mutagenic carcinogen induced tumors in p53 haploinsufficient mice showed that chromosome 11 loss of heterozygosity, including loss of a functional p53 allele, through mitotic recombination and chromosome mis-segregation

Development and use of rodent models for identification of hazards in our environment are critical to preventing and/or minimize human exposure to toxic substances. However extrapolation from rodents to humans is difficult. One way to help reduce uncertainty over the use of rodent models as surrogates is to establish that the mechanisms of toxicity and/or carcinogenicity are similar between the two species. These mechanism are similar between rodents and humans and assist in the risk assessment process and identify those substances to which exposure should be eliminated or minimized to the fullest extent possible.

Boley, S. E., Anderson, E. E., French, J. E., Donehower, L. A., Walker, D. B., and Recio, L. (2000) Loss of p53 in benzene-induced thymic lymphomas in p53+/- mice: evidence of homologous recombination. Cancer Res. 60: 2831-2835.

French, J., Lacks, G., Trempus, C., Dunnick, J., Mahler, J., Foley, J., Tice, R., and Tennant, R. (2001) Loss of heterozygosity frequency at the *Trp53* locus in p53 haploinsufficient mice is carcinogen and tissue dependent, Carcinogenesis 22: 98-106.

Hulla, J.E., French, J.E., and Dunnick, J.K. (2001) Chromosome 11 loss from thymic lymphomas induced in heterozygous *Trp53* mice by phenolphthalein reveals mitotic recombination. Toxicol. Sci, 60: 264-70.

Hulla, J. E.., French, J. E., and Dunnick, J. K. (2001) Chromosome 11 allelotypes reflect mechanism of chemical carcinogenesis in heterozygous p53-deficient mice Carcinogenesis 22: 89-98.

Development of new drugs to combat cancer and associated pain.

New synthetic opioid drugs were developed that could be applied to alleviating cancer and post-operative pain. Selective compounds were shown to inhibit a protein on the surface of some cancer cells that makes them resistant to chemotherapy regimes. These opioid substances should represent a new approach as specific chemosensitizing agents. Other substances are being developed to combat drug addiction with causing dependency.

Okada, Y., Fukumizu, A., Takahashi, M., Shimizu, Y., Tsuda, Y., Yokoi, T., Bryant, S. D., and Lazarus, L. H. (2000) Synthesis of stereoisomeric analogues of endomorphin-2, H-Tyr-Pro-Phe-Phe-NH2, and examination of their opioid receptor binding activities and solution conformation. Biochim. Biophys. Res. Commun. 276: 7-11, 2000.

Labarre, M, Butterworth, J., St-Onge, S., Payza, K., Schmidhammer, H., Salvadori, S., Balboni, G., Guerrini, R., Bryant, S. D., and Lazarus, L. H. (2000) Inverse agonism by Dmt-Tic analogues and HS 378, a naltrindole analogue. Eur. J. Pharmacol. 406: R1-R3.

Santagada, V., Balboni, G., Caliendo, G., Guerrini, R., Salvadori, S., Bianchi, C., Bryant, S. D., and Lazarus, L.H. (2000) Assessment of substitution in the second pharmacophore of Dmt-Tic analogues. Bioorg. Med. Chem. Lett. 10, 2745-2748.

Lovekamp, T., Cooper, P.S., Hardison, J., Bryant, S.D., Guerrini, R., Balboni, G., Salvadori, S., and Lazarus, L. H. (2001) Inhibition of human multidrug resistance P-glycoprotein-1 by analogues of a potent δ-opioid antagonist. Brain Res. 902: 131-134.

Santagada, V., Fiorino, F., Severino, B., Salvadori, S., Lazarus, L. H., Bryant, S.D., and Caliendo, G. (2001) A convenient synthesis of N-Fmoc-N,N'-bis-Boc-7-guanyl-1,2,3,4-tetrahydro-3-isoquinoline carboxylic acid (Fmoc-N,N'-bis-Boc-7-guanyl-Tic-OH, GTIC). Tetrahedron Lett. 42: 3507-3509.

Ingman, K., Salvadori, S., Lazarus, L., Korpi E. R., and Honkanen, A. (2001) Selective d-opioid receptor antagonist N,N(CH3)2-Dmt-Tic-OH does not reduce ethanol intake in alcohol-preferrring AA rats. Alcohol, in press.

The carcinogenic potential of nitroaromatic chemicals is related to structure.

NTP has studied the carcinogenic potential of two high-production chemicals, o-nitrotoluene and p-nitrotoluene. The nitrotoluenes are used to synthesize agricultural and rubber chemicals, and dyes. More than 30 million pounds of o-nitrotoluene are produced each year and more than 10 million pounds of p-nitrotoluene are produced. The o-nitrotoluene isomer showed clear evidence of carcinogenic activity causing mesotheliomas, subcutaneous skin neoplasms, mammary gland

fibroadenomas and liver neoplasms in male rats; subcutaneous skin neoplasms and mammary gland fibroadenomas in female rats; hemangiosarcomas and carcinomas of the large intestine (cecum) in male and female mice; and hepatocellular neoplasms in female mice. In a stop-study in male rats, the chemical was administered for three months, and the animals were then continued on study without dosing for up to two years. There was clear evidence for carcinogenic activity in these male rats, including mesotheliomas, skin neoplasms, and mammary gland neoplasms. These studies showed that the critical events leading to tumor formation occurred early in the study, and this damage was irreversible.

There was no clear evidence of carcinogenic activity in the p-nitrotoluene studies. P-nitrotoluene caused equivocal evidence of carcinogenic activity in male rats (skin neoplasms); some evidence of carcinogenic activity of in female rats (clitoral gland neoplasms); equivocal evidence of carcinogenic activity in male mice (lung neoplasms); and no evidence of carcinogenic activity in female mice. Aromatic chemicals with an ortho-substitution are more likely to be carcinogenic than aromatic chemicals with the substitution in the meta or para positions.

NTP Technical Report on the Toxicology and Carcinogenesis studies of o-nitrotoluene in F344/N rats and B6C3F1 mice. NTP TR 504, NIH Publication 01-4438. NIEHS Research Triangle Park, NC. 2001.

NTP Technical Report on the Toxicology and Carcinogenesis studies of p-nitrotoluene in F344/N rats and B6C3F1 mice. NTP TR 498, NIH Publication 01-4432. NIEHS Research Triangle Park, NC. 2001.

HIGHLIGHTS FROM THE NATIONAL TOXICOLOGY PROGRAM (NTP)
September 2001

Environmental Toxicology Program Retreat

The Environmental Toxicology Program (ETP) held a retreat, August 21-23, 2001, at the Hilton Riverside Hotel in Wilmington, North Carolina. A primary focus was strategic planning for the ETP/NTP so the program can position itself to meet current and future challenges facing public health. Plenary lectures included talks and discussion about issues within public health, the current status of ETP/NTP toxicology research and testing, and information about new technologies and how they might be useful for the ETP/NTP. In small breakout groups, attendees discussed in greater detail future directions and how new technologies might be integrated into research and testing for specifically targeted areas.

Lesions of Genetically Altered Mice

The NTP has an interest in alternative animal models and has been involved in evaluating transgenic mouse models. A CD, *Lesions of Genetically Altered Mice,* has been compiled that contains characteristic lesions seen in Tg.AC, p53+/-, p53 x Tg.AC, ERKO, APC-min, and TRAMP mice in addition to liver lesions from two bitransgenic mice. Relevant literature references accompany the histopathologic images. Copies are available from the Laboratory of Experimental Pathology, NIEHS (Contact: Sheila Withers-Gibbs at WITHERSG@niehs.nih.gov).

Agent Orange

Scientists representing the United States and Vietnamese government met in Hanoi, Vietnam in July 2001 to continue discussions begun in November 2000 regarding collaborative research on the health and environmental effects of Agent Orange and its principal toxic contaminant, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (dioxin). Dr. Christopher Portier, Director of the Environmental Toxicology Program, NIEHS and Associate Director of the NTP, led the U.S. delegation that included scientists from the U.S. Environmental Protection Agency and the Centers for Disease Control and Prevention. Dr. Nguyen Ngoc Sinh, General Director of the National Environmental Agency in Hanoi, led the Vietnamese delegation that included scientists from the National Centre for Natural Science and Technology.

Two projects were agreed upon:

- a joint Vietnam-United States scientific conference on human health and environmental effects of Agent Orange/Dioxin tentatively planned for April 2002
- a pilot study for screening dioxins in soil and sediments.

NTP Board of Scientific Counselors Technical Reports Review Subcommittee

The NTP conducts research studies that are designed to characterize and evaluate the toxicological potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The NTP Board of Scientific Counselors Technical Reports Review Subcommittee evaluates the results from the studies in an open, peer-review meeting. The next meeting of the Subcommittee is scheduled for October 18, 2001 at the NIEHS. The

candidate reports scheduled for consideration are the two-year bioassays on vanadium pentoxide, riddelliine, and 2,4-hexadienal, and the short-term toxicity studies on diazoaminobenzene.

NTP Chemical Nomination and Selection

The NTP openly solicits nominations of chemicals and substances for study and receives nominations from a variety of groups including Federal agencies, public, industry, and labor unions. The NTP welcomes public comments on nominations and information from toxicology and carcinogenesis studies, as well as supplementary data on current production levels, human exposure, use patterns, or environmental occurrence. Nominations for NTP studies undergo several levels of review before toxicological studies are designed and implemented. The following nominated chemicals and substances completed the review process in 2001 and attention is being given for their study by the NTP.

Aluminum complexes found in drinking water 1. Aluminum fluoride 2. Aluminum citrate	Lemon oil and Lime oil
Bilberry fruit extract	Local anesthetics that metabolize to 2,6-xylidine or o-toluidine 5. Bupivacaine 6. Prilocaine
Black cohosh	Microcystin-LR
Blue-green algae (dietary supplements and selected toxins)	Organotins occurring in drinking water 5. Monomethyltin trichloride 6. Dimethyl dichloride 7. Monobutyltin trichloride 8. Dibutyltin dichloride
Cefuroxime	All-*trans*-retinyl palmitate
Clarithromycin	S-Adenosylmethionine
D&C red no. 27 and D&C red no. 28	Senna
N,N-Dimethyl-*p*-toluidine	

Centers

NTP Center for the Evaluation of Risks to Human Reproduction (CERHR)

The next chemical planned for evaluation by the Center is methanol (CAS No. 67-56-1). An expert panel meeting is tentatively scheduled for October 15-17, 2001 at the Radisson Hotel Old Town, Alexandria, VA. The draft expert panel report is currently available on the CERHR web site (http://cerhr.niehs.nih.gov); public comment is being solicited on the draft report. A large toxicity database exists on the reproductive and developmental effects of methanol. Methanol is a commercially important, high production volume chemical (10.54 billion pounds, US production, 1993), with potential for occupational, consumer, and environmental exposure.

NTP Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

NICEATM and the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM) collaborate to develop, validate, and achieve regulatory acceptance of new and improved test methods. The NTP Advisory Committee on Alternative Toxicological Methods will meet September 25, 2001 at the NIEHS. This committee provides advice on the activities and priorities of NICEATM and ICCVAM and on ways to

foster partnership activities and productive interactions among all stakeholders. The Committee will discuss recent ICCVAM/NICEATM test method meetings and proposed future activities. Meeting minutes will be available on the ICCVAM/NICEATM web site (http://iccvam.niehs.nih.gov). This meeting is open to the public with time set aside for public comment.

NTP Exhibits

The NTP continues to work toward increasing public outreach about its programs and activities.

- The NTP participated with an exhibit July 8-12, 2001, at the 9th International Congress of Toxicology meeting in Brisbane, Australia.
- The NTP is scheduled to have an exhibit at the 129th Annual Meeting of the American Public Health Association, October 21-25, 2001 in Atlanta, GA. The NIEHS/NTP is sponsoring a session, *Genomics and Environmental Health: Enhancing the Public Health Impact of Research Program of the National Institute of Environmental Health Sciences and the National Toxicology Program,* on Wednesday, October 24, 4:30-6 PM. Additional details about this session are available at http://apha.confex.com/apha/129am/techprogram/session_7254.